In Silico Molecular Docking of Marine Drugs Against Cancer Proteins

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Abstract

At present, the criteria used to select optimal new anticancer drug candidates include inhibitors of cell proliferation, essential reaction and pathways in cancerous cells. In silico approach resulting in the identification of essential reactions and pathways spreads across several parts of metabolism. The aim of our study is to study the interaction of broad spectrum antibiotic squalamine and LAQ824 with 4 selected anticancer drug target enzymes in Silico molecular docking approach. The ligand squalamine showed minimum binding energy −6.88 kcal/mol with promyleocytic leukemia (PDB ID-1BOR) and −5.68 kcal/mol with estrogen related receptor α (PDB ID-1XB7). Similarly, the compound LAQ824 showed minimum binding energy -6.77 kcal/mol with BRCA2 (PDB ID-1NOW). The compound squalamine interacted with several amino acid residues, of which glutamate was found to be common among all the target enzymes for protein and hydrogen bond formation. Likewise, lysine was found to be common among all the target enzymes for protein and hydrogen bond formation with LAQ824. Results of our study suggested that molecular docking approach could be a potential tool to identify the hydrogen bond interactions and the molecular mechanisms of diseases. It was concluded that squalamine and LAQ824 ligands would be of potent drug targets to treat various cancers based on the docking approach.

Keywords

Molecular Docking, Squealing; LAQ824; Cancer Proteins

Introduction

Victims of cancer has significantly risen over past few decades as the advancements in medical research is still in progress to rise above this immune compromised condition. Cancer is the most frequently diagnosed and major leading cause of death. There are more than 100 types of cancer characterized with abnormal cell growth. During the early days of targeted cancer chemotherapy, the novel compounds were designed to target one single crucial oncoprotein in a highly specific fashion. Nowadays, being a multifactorial disease, cancer has been recognized with multilevel cross-stimulation among the targets along several pathways of signal transduction that finally led to neoplasia (Zdrazil and Ecker, 2010). Thus, by blocking only one of these pathways, the other pathways involved in the manifestation of cancer (which are not blocked) could act as salvage mechanism for the cancer cell. Thus, a second generation of so-called "multitargeted" chemotherapeutics aims at the interference of a multitude of these pathways/oncoproteins that is expected to result in a broader antitumor effect.

Different types of tubulin proteins involved in different types of cancers are sensitive to drug molecules. The anticancer agents that currently available for the treatment of cancer are narrow minded. The major cause of limited success of chemotherapy is the acquisition of drug resistance by tumors, inadequate target drug delivery due to abnormal tumor vasculature, and its toxicity (Jackson, 2012). Hence there is a need of innovative research that may identify novel molecule as a target for development of therapeutic agents.

In silico modeling is the bypass for the traditional drug testing compounds, synthesized in time consuming multi step process against biological screens. It is the new approach to clinical chemistry for the optimization of screening and testing by means of the observation on particular compound (Waterweed and Gifford, 2003). The need of biological screening and chemical synthesis has increased in order to obtain the early information of absorption, distribution, metabolism, excretion, and toxicity data.

An increasing number of protein crystallographic structures are becoming available based on high throughput structural genomics projects thus prediction of a potential lead and its potential target is a fundamental step in order to investigate the molecular recognition mechanisms of protein (Abagyan and Totrov, 2001).

Proteins related to breast cancer and leukemia was targeted as macromolecules i.e. promyleocytic leukemia (PDB ID-1BOR), estrogen related receptor α (PDB ID-1XB7) and BRCA2 (PDB ID-1NOW).

Breast cancer is the most frequently diagnosed and the second leading cause of cancer for women. As angiogenesis is one of the crucial steps in the pathogenesis of tumors (Mudusudan and Haris, 2002), identification of novel compound for development of therapeutic agent need to be performed that will stop the early steps in metastasis.

Marine drugs have been the source of novel bioactive compounds and the criteria used for selection of anticancer drugs includes the inhibitors of cell proliferation of cancerous cells, tumor formation and rapid growth (Mancini et al., 2007). A strong naturally derived broad-spectrum antibiotic Squalamine has been reported to be derived predominantly from shark species (Moore et al., 1993) can perform a powerful anti-angiogenesis function. Because of its potent anti-angiogenic effects, squalamine also shows considerable promise in the treatment of solid tumors such as ovarian cancer. LAQ824 potential of bioactive molecules from marine sponges *Psammaplysilla* sp. is known for inhibition of histone deacetylase (HDAC) (Beloueche-Babari et al., 2012). This Psammaplin derivative is proved to be a promising agent to cure cancer.

Leukemia is also a type of cancer that starts in blood forming tissue such as the bone marrow and causes large number of abnormal blood cells to be produced and enter the blood. In acute leukemia's, histone deacetylase (HDAC) inhibitors exert their antitumor effects by relieving transcriptional repression and thereby reversing the differentiation arrest induced by chimeric oncogenes (Minucci and Pelicci, 2006).

Our study dealt with the examination of the interactions between marine based novel compound and cancer protein in silico by molecular docking method in order to calculate the minimum binding energy (kcal/mol) between them. Molecular docking determines the binding affinity and activity of small molecules, which aims to determine the 3D conformation and binding interactions.

Materials and Methods

The ligands squalamine and LAQ824 were extracted from Squalus acanthias and Psammaplysilla sp. structure was drawn in chemdraw software and the chemdraw format of the ligand was then converted to PDB format. Squalus acanthias belongs to a family squalidae which is a small demersal shark of temperate continental shelf seas worldwide. Psammaplysilla sp. is a marine sponge widely distributed in ocean beds.

Target Enzymes

Four drug resistant enzymes were selected as targets for docking studies and their 3D structures were downloaded from the Protein Data Bank (PDB). The water molecules were removed during modeling. The standard protocol and modeling methods were followed for docking studies. The energy minimized protein structure was included prior to docking to accommodate hydrogen atoms and which were not included in the crystal structure coordinates.

Molecular Docking Stimulation

In silico virtual screening of receptors is, however, a daunting task, for both of the receptor based approaches (docking) and ligand based approaches (Squalamine, LAQ824). Two goals involved in docking studies are to quickly determine the most likely binding mode of leads and to measure the income of its expected binding affinity for the target protein.

Estimation of ligand protein affinity/ ligand–DNA or drug target affinity is one of the major and the basic steps in drug discovery. Only the potential molecules are taken up further for the analysis which demonstrates a desirable binding for the targeted receptor. To perform the docking model, the Auto Dock 4.0 suite molecular-docking tool was used and the methodology was followed (Gowthaman et al., 2008). The anti-cancer compounds were manually docked into sites of the enzymes and the docking energy was monitored to achieve a minimum value. The default parameters of the automatic settings were used. Each docking experiment consisted of 10 docking runs and the search was conducted in a grid of 40 points per dimension. The binding position and bound conformation of the peptide, and the rough estimate of its interactions were examined with the Auto Dock results. To analyze the mode of binding docked conformation with minimum binding energy was selected.

Results and Discussion

A molecular docking simulation study was undertaken to investigate and to access the binding

efficiency of squalamine and LAQ824 with the anticancer target proteins. Docking of squalamine (ligand) with the active site of cancer drug target enzymes, PML (Fig. 1 & 2) and ERR alpha (Fig. 3 & 4) resulted in 10 docked confirmations.

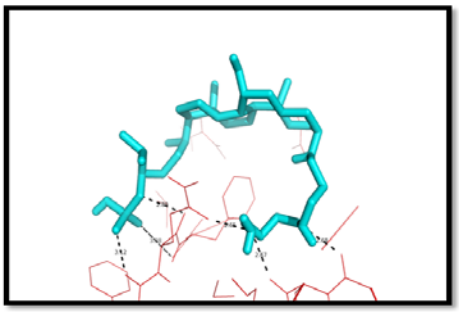

FIG. 1 IN SILICO BINDING OF PML WITH SQUALAMINE. (BINDING ENERGY - 4.31 KCAL/MOL, RECEPTOR CONTACTS- GLY37, MET38, GLN5, PHE4, PHE6)

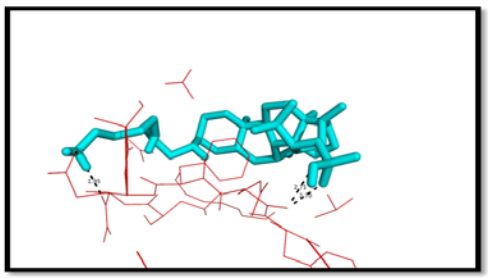

FIG. 2 IN SILICO BINDING OF PML WITH SQUALAMINE (BINDING ENERGY - 6.88 KCAL/MOL, RECEPTOR CONTACTS- GLU3, PRO23, GLN5)

FIG. 3 IN SILICO BINDING OF ERR-A WITH SQUALAMINE. (BINDING ENERGY - 0.97 KCAL/MOL, RECEPTOR CONTACTS- GLU303, GLU303)

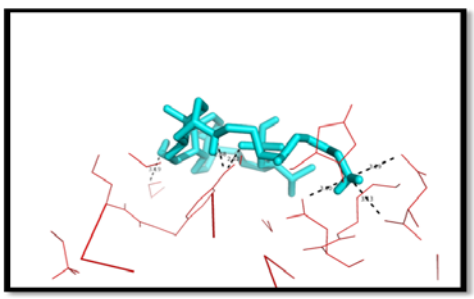

FIG. 4 IN SILICO BINDING OF ERR-A WITH SQUALAMINE. (BINDING ENERGY - 5.68 KCAL/MOL, RECEPTOR CONTACTS- ASP326, ASP326, ASP329, TYR213, THR216)

The results of Auto Dock 4.0 were verified by considering some top clusters of conformations in addition to the best scored one. The docking accuracy was evaluated in terms of the root mean square deviation (RMSD) and the prediction was considered successful if the RMSD value was less than 2.0˚A for the best scored conformation (Kroemer, 2007). The docking results are ranked according to least binding energies for each of the investigated enzymes. Same procedure was carried out using LAQ824 as a ligand and BRCA2 as a cancer drug target enzyme. Different enzymes were selected on the basis of their mode of action. Between the 2 enzymes screened promyleocytic leukemia protein (PML) showed the binding energy of −6.88 kcal/mol (Table 1) and hence showing maximum interaction with the enzyme responsible for blocking the differentiation pathway, in cancerous cell leading to apoptosis. Similarly interaction between BRCA2 (macromolecule) and LAQ824 (ligand) showed the binding energy of -6.77 kcal/mol (Fig. 5)

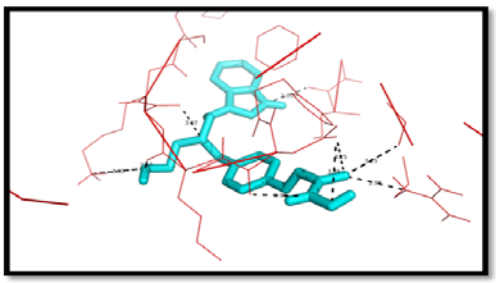

FIG. 5 IN SILICO BINDING OF BRCA2 WITH LAQ824. (BINDING ENERGY - 6.77 KCAL/MOL, RECEPTOR CONTACTS- SER439, GLU432, ARG435, ASP410, LYS412, ALA413, LYS414)

The compound squalamine interacted with GLY37, MET38, GLN5, PHE4, and PH6 of the PML protein (Table 1). Likewise compound LAQ824 interacted with SER439, GLU432, ARG435, ASP410, LYS412, ALA413, LYS414 of BRCA2 protein (Table 1).

Squalamine, a natural aminosterol, has been previously reported with much biological activity. In a rat model of chronic P. aeruginosa pneumonia, treatment twice daily with a squalamine aerosol for 6 days leads to a significant reduction in the pulmonary bacterial count (Hraiech et al., 2012) which also possessed fungicidal property. It acts on yeast by disrupting the outer membrane and it has significant in vitro activity against Trichophyton and Microsporum sp. The minimum inhibitory concentrations (MICs) ranged from 4-16 mg/l for squalamine against these superficial dermatophyte infections (Coulibaly et al., 2013). Squalamine exhibits broad-spectrum antiviral activity against human pathogens which were studied in vitro as well as in vivo. Both RNA- and DNA-enveloped viruses were shown to be susceptible. HDAC inhibitor LAQ824 has

a greater activity against tumour cells which are all resistant to chemotherapy (Zasloff et al., 2011). Several studies have reported the significance use of LAQ824 which exhibited greater induction of apoptosis in vitro and in vivo tumour cells with combination therapy.

TABLE 1: MOLECULAR DOCKING OF SQUALAMINE AND LAQ824 WITH VARIOUS DRUG TARGET ENZYMES

Target molecule	PDB ID	Ligand	Binding energy (kcal/mol)	Receptor contact	Vander walls energy
PML	1BOR (complex 3)	Squalamine	-4.31	GLY37, MET38, GLN5, PHE4, PHE6	-8.96
PML	1BOR (complex 10)	Squalamine	-6.88	GLU3, PRO23, GLN5	-9.97
ERR-α	1XB7 (complex 10)	Squalamine	-0.97	GLU303, GLU303	-6.44
ERR-α	1XB7 (complex 3)	Squalamine	-5.68	ASP326, ASP326, ASP329, TYR213, THR216	-8.19
BRCA2	1NOW	LAQ824	-6.77	SER439, GLU432, ARG435, ASP410, LYS412, ALA413, LYS414	-9.62

Conclusions

The results of docking study showed that the active sites of enzymes can accommodate (squalamine and LAQ824) different sizes and structures, adopting a variety of binding modes and interactions which is demonstrated by the number of structurally diverse scaffolds.

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